part in this process vary strongly depending on the organism. Examples of such proteins are light harvesting complexes such as LH2 and LH1, which are part of purple bacterial photosynthetic membranes, or bacteriorhodopsin, a membrane protein which uses sunlight directly to generate a proton gradient. To fully understand the underlying mechanism of these proteins, the analysis and simulation of the exciton transfer as well as the proton and electron transfer processes are crucial. We present here the application of a Dynamic Monte-Carlo (DMC) algorithm [1] to simulate this kind of transfer kinetics [2]. At each time step the analyzed system is represented by a microstate description [3]. Depending on the kind of reaction, transition rates between these states are taken either from the literature or are calculated based on continuum electrostatics and Marcus theory. To test the reliability of our method energy transfer in arrays of light harvesting antenna complexes (LH2 [4,5]), transfer kinetics in LH1-RC complexes and proton transfer in bacteriorhodopsin were investigated.

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S9.P15

A computer simulator of mammalian cytochrome *c* oxidase activity

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Cytochrome c oxidase is a well-known terminal enzyme complex of mitochondrial respiratory chain. It provides electron transport between several redox-centers which belongs to protein subunits from reduced cytochrome C to oxygen. At the same time the protein implements transmembrane proton pumping. This property makes one possible to consider cytochrome c oxidase as a primary electro-chemical potential generator and supply energy needs for ATP synthesis. The catalytic cycle of cytochrome c oxidase can be represented in terms of consecutive transition between distinct states with an increasing number of electrons transferred to the catalytic site (haem a3-CuB). This process is usually described in terms of kinetic constants and it is tempting to model cytochrome c oxidase turnover in common enzyme-kinetic approach. However, the whole set of measured kinetic parameters of the cycle is unavailable whereas some local processes and protein structure have been defined in details. At the present study we introduce a novel computer simulator of cytochrome oxidase activity. This digital mimic is based on algorithmic programming of electron and proton transfer between the fixed centers. Thus the enzyme activity is modeled for a single protein explicitly. The simulator makes it possible to evaluate the ratio between pumped protons and transfers electrons and it is varied from 0.45 to 0.85 under different external parameters. The advantage of the introduced approach is a possibility to extend and modify the processes within the enzyme according to new evidences of the protein structure.

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S9.P16

Sulfide complex formation and redox interactions with heme enzymes

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Sulfide (H_2S) forms reversible low spin complexes with ferric myoglobin and hemoglobin and is also a potent inhibitor of cytochrome c oxidase. Some hemoglobins from high sulfide environment organisms are also sulfide reducible, as is the mammalian oxidase when in its oxidized 'pulsed' state. In the presence of oxygen the resulting oxidase mixed valence (partially reduced) species then generates a higher oxidation state, compound 'P', and concurrently oxidizes H_2S to sulfane/persulfide species. Classical studies of eukaryotic catalase and plant peroxidases indicated that H_2S inhibits these enzymes both by reversible binding to the ground (ferric) state and by quasi-irreversible reactions with ferryl states which form covalent 'sulf' derivatives. But, unlike its behavior with metmyoglobin and cytochrome c oxidase, H_2S does not form low spin complexes with the ferric haems of the hydroperoxidases at room temperature. Instead a more remote iron-ligand binding occurs, creating high spin complexes (as determined by UV–visible spectrophotometry) similar to those formed by reaction with some carboxylic acid anions (acetate and formate). In contrast EPR analysis at 10 K does show the presence of multiple low spin species in the plant (horse radish) peroxidase sulfide complex and a mixture of high and low spin forms in sulfide-treated catalase. This variability in ligation chemistry may influence the balance between reversible heme (Fe) binding and heme reduction by sulfide and hence modulate its proposed gasotransmitter physiological functioning. A model and rationale for these complex reaction sequences will be presented. This research was supported by a Leverhulme Trust grant to CEC.

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S9.P17

Exploring O_2 diffusion in A-type cytochrome *C* oxidases: MD simulations uncover two alternative channels towards the binuclear site

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Cytochrome *c* oxidases (CCOX) are members of the heme–copper oxidase superfamily and they are the terminal enzymes of the respiratory

chain. These proteins are membrane-bound multi-subunit redox-driven proton pumps, which couple the reduction of molecular dioxygen to water with the creation of a transmembrane electrochemical proton gradient. Over the last 20 years, most of the CCOX research focused on the mechanisms and energetics of reduction and/or proton pumping and little emphasis has been given to the pathways used by dioxygen to reach the binuclear site. The main objective of this work is to identify possible alternative dioxygen pathways in the reduced CCOX from *Rhodobacter sphaeroides* [1] using extensive Molecular Dynamics (MD) simulations. Our simulations allowed the identification of three possible dioxygen channels, all starting in the membrane hydrophobic region and connecting the surface of the protein to the BNC. One of these channels corresponds to the pathway inferred from the X-ray data available [2], whereas the other two are alternative routes for O_2 to reach the BNC. Both alternative channels start in the membrane spanning region and terminate close to Y288I (which is covalently linked to the H284I imidazole group).

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S9.P18

Constructing new proton pathways in nitric oxide reductases

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Bacterial nitric oxide reductases (NORs) are members of the heme–copper oxidase (HCO) superfamily and are evolutionarily related to the oxygen-reducing cytochrome oxidases. The first crystal structure of the cytochrome c-dependent NOR (cNOR) [1], supported by the molecular dynamics simulations [2] and mutagenesis studies [3–4] suggested that protons for NO reduction are supplied from the periplasm. Somewhat surprisingly, a structure of the quinol-dependent NOR (qNOR) [5] showed a water channel from the cytoplasm that functions as a pathway for proton delivery to the active site. Interestingly, the water channel is positioned equivalently to the K-pathway in oxidases. We will present preliminary results of a study that aims to construct the water channel (and possibly a new functional proton pathway) in the corresponding region in cNOR. The molecular dynamics simulations performed for several cNOR mutants showed remarkable formation of a new water channel, within 5–50 ns of simulation time. The project could shed light on development of proton pathways in the HCO superfamily.

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S9.P19

ATP dependent inhibition of cytochrome *c* oxidase results in decreased ROS production

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More than 15 years ago, the second mechanism of mitochondrial respiratory control was proposed based on the allosteric inhibition of cytochrome c oxidase (CytOx) activity by ATP. This was proposed to be physiologically most important regulation of this enzyme, and also of mitochondrial respiration. It was 'suggested' that this mechanism keeps the mitochondrial ROS concentrations under low healthy values [1] by regulating the mitochondrial respiration and membrane potential [2].

Previously, it was difficult to show the correlation between 'kinetics' of CytOx activity and mitochondrial membrane potential together with ROS production since methodological variations during kinetics measurements of CytOx activity are altered especially by the presence of a detergent. Here, in this study we first tried to optimize the conditions for measuring the CytOx kinetics in intact rat heart mitochondria (without detergent). This was an absolutely essential step to see the change in mitochondrial membrane potential and ROS concentrations under the measuring conditions of CytOx kinetics. Rat heart mitochondria were isolated by standard procedure of isolation. Protein estimation was performed by BCA method. In kinetics studies, oxygen consumption by CytOx in intact mitochondria was measured polarographically. Changes in mitochondrial membrane potential and ROS concentrations were detected by DIOC6(3) and MitoSOX, respectively in fluorescence-based assays. For the first time, a direct correlation of CytOx kinetics studies to the concentrations of ROS in intact mitochondria is shown. We found a sharp decrease in the ROS concentrations when the activity of CytOx is measured in the presence of ATP and regenerating system (phosphoenolpyruvate + pyruvate kinase). Moreover, in the presence of allosteric ATP inhibition of CytOx, lowest concentrations of ROS were measured in intact rat heart mitochondria.

ATP dependent inhibition of CytOx activity is crucial for mitochondrial bioenergetics as it maintains the redox balance by keeping the ROS concentrations at low values.